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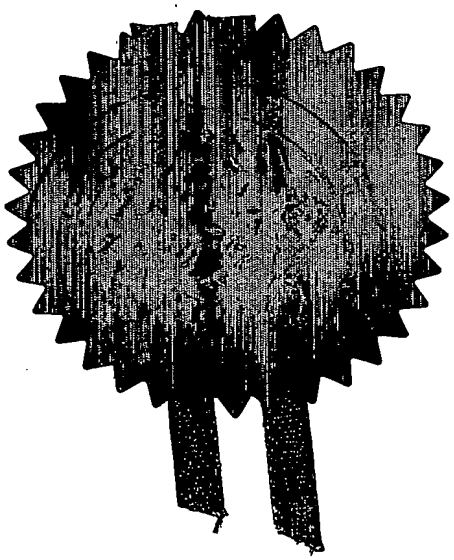

Signed Stephen Hordley

Dated 5 May 2004

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27MAR03 E795539-1 D00085
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Request for grant of a patent

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1. Your reference — JMH/8153

2. Patent application number *(The Patent Office will fill in this part)* — 0307011.7

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

RegenTec Ltd
1 Faraday Building
Nottingham Science & Technology Park
University Boulevard
Nottingham NG7 2QP

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom — 8597791001

4. Title of the invention — Porous Matrix

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Stevens Hewlett & Perkins
1 St. Augustine's Place
Bristol BS1 4UD
UK

Patents ADP number *(if you know it)* — 1545002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	10
Claim(s)	5
Abstract	-
Drawing(s)	-

10. If you are also filing any of the following, state how many against each item.

Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination and search *(Patents Form 9/77)*	1
Request for substantive examination *(Patents Form 10/77)*	1
Any other documents *(please specify)*	-

11. I/We request the grant of a patent on the basis of this application.

Signature Stevens Hewlett & Perkins Date 27/03/03

Stevens Hewlett & Perkins

12. Name and daytime telephone number of person to contact in the United Kingdom J M HEATON 0117 9226007

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POROUS MATRIX

This invention relates to a porous matrix. More particularly, the present invention relates to porous matrices which are intended to be used in the animal body and which are formed *in situ* at a target tissue site.

Many patent applications describe the use of gels or sols, especially hydrogels for use as tissue scaffolds. For example, WO 00/ 23054 describes the use of polyvinyl alcohol microspheres in the occlusion of blood vessels or embolizations. WO 99/ 15211 and WO 00/ 64977 describe the use of hydrogels as a tissue scaffold. The hydrogels are implanted into a patient in order to support tissue growth and or repair.

The use of hydrogels as tissue scaffolding is problematical in that although the gels themselves may adequately fill the cavity in to which they are inserted, they have poor diffusion properties and as such drugs, nutrients or other factors to be supplied to the gel do not adequately diffuse through the gel. This problem is exacerbated where the gel is seeded with living cells since the poor diffusion of nutrients can lead to premature cell death possibly resulting in failure of the treatment. A further problem associated with gel scaffolds is that the cross-linking methods used to stabilise or solidify the gels, especially *in situ*, can damage the entrapped cells.

Scaffolds based on water-insoluble polymers are also known in the art, for example WO 99/ 25391 describes the use of poly (lactide-co-glycolide) (PLGA) as a polymer scaffold for the regeneration of tissue, especially bone tissue. The polymers are processed so as to form a porous structure. As with the hydrogels, the water-insoluble polymers are implanted into a patient in order to support tissue growth and or repair.

However, the disadvantage of such water-insoluble polymers is that they can only fill cavities with an open shape and methods of shaping the materials are yet to be perfected. Additionally, where the scaffold is to be seeded with cells, the seeding is inefficient (few pores are filled with cells) or the cells are damaged by the structure during the seeding process, and the surrounding tissue cells may also be damaged by the implantation procedures.

WO 99/11196 describes the use of a particulate matrix as tissue scaffold, the particles having internal cross-linking to stabilise the structure of the particle.

Similarly, PCT/GB02/02813 describes an open porous matrix of particulate material for *in vivo* use in or on a target tissue in medicine, the matrix comprising particles cross-linked with one another so as to define pores there between.

The present invention provides a process for the production of a porous matrix, the process comprising the steps of:

- bringing a first, carrier phase into a fluid state,
- introducing a second, suspended phase into the first phase,
- mixing the first and second phases to ensure the required distribution of the second phase through the first phase is achieved, and
- allowing the first phase to solidify, with the second phase entrained therein.

The term "fluid" as used herein is intended to define any substance which flows and therefore includes liquids, gases and solids (e.g. in powder or granule or grain form) which are able to flow and to conform to the outline of their container.

The first carrier phase may not be a wholly liquid state but is rendered sufficiently fluid to mix with and carry the second phase. For example, the carrier phase may be fluid but tacky and coat the particulate material of the second phase. Alternatively, both the first and second phases may be in particulate or powder form and mixed together. In this case it is again desirable that the first phase is tacky or otherwise able to coat any particulate material of the second phase.

Preferably, the first phase transforms from a fluid state to a solid or semi-solid state on the change of a single parameter, for example temperature, pH, introduction of a setting agent, presence/absence of light, ultra-violet curing or under anaerobic conditions. Most preferably, the first phase transforms due to a change in temperature or in pH. Where

temperature is used it is preferred that the temperature is sufficient to render the phase workable but not damage the surrounding tissues when used. A pre-use sintering step may be applied to either phase.

The second phase is preferably a solid phase, but a further liquid phase may be used, especially where the liquid is an emulsion or suspension of particulate material. Where the porous matrix is to be used as a tissue scaffolding matrix, the second phase preferably contains cells for the formation of new tissue.

Accordingly, the present invention further provides a tissue scaffolding matrix, the matrix comprising a first, carrier phase and a second, suspended phase contained within the first phase. Preferably, the tissue scaffolding matrix is prepared according to the abovedescribed method.

The first and second phases may be made from similar materials, with different solidifying properties. For example, the first and second phases may be made from similar polymers with different gelling pHs or different melting temperatures or glass transition points.

Generally, the phases of the invention will comprise polymers. Examples of polymers usable in the present invention include poly-lactide, poly-glycolide, poly-lactide poly-glycolide copolymers, poly-lactide polyethylene glycol (PEG) copolymers, polyethylene glycol polypropylene block co-polymers for example that sold under the trade name Pluronics F127™, natural or synthetic polymers such as silk, elastin, chitin, chitosan, fibrin, fibrinogen, poly(α-hydroxyacids) especially polylactic or polyglycolic acids, poly (anhydrides) or poly(ortho esters). Polymers of polyhydroxy acids including polyhydroxybutyric acid, lactic, glycolic and s-caproic acid, polyanhydrides, polyorthoesters, polyphosphazenes, polyphosphates, polycaprolactone. Co-polymers prepared from the monomers of these polymers may also be used as may random blends of these polymers or mixtures or combinations thereof.

Similarly, synthetic biodegradable polymers may be used, examples of which are set out in list form below:

1. Polyesters including:
poly(lactic acid)
poly(glycolic acid)
copolymers of lactic and glycolic acid
copolymers of lactic and glycolic acid with polyethylene glycol)
poly (E-caprolactone)
poly (3-hydroxybutyrate)
poly (p-dioxanone)
polypropylene fumarate)

2. Poly (ortho esters) including:
Polyol/diketene acetals addition polymers (as described by Heller ACS Symposium Series 567,292-305, 1994).

3. Polyanhydrides including:
poly (sebacic anhydride) (PSA)
poly (carboxybiscarboxyphenoxyphenoxyhexane)(PCPP)
poly [bis (p-carboxyphenoxy) methane](PCPM)
copolymers of SA, CPP and CPM 9as described by Tamada and Langer in Journal of Biomaterials Science Polymer Edition, 3,315-353,1992 and by Domb in Chapter 8 of the Handbook of Biodegradable Polymers, ed. Domb A. J. and Wiseman R. M., Harwood Academic Publishers).

4. Poly (amino acids)

5. Poly (pseudo amino acids) (Including those described by James and Kohn at pages 389-403 of Controlled Drug Delivery Challenges and Strategies, American Chemical Society, Washington DC).

6. Polyphosphazenes including:
derivatives of poly [(dichloro) phosphazene]
poly [(organo) phosphazenes]
polymers described by Schacht in Biotechnology and Bioengineering, 52,102-108,1996.

In a preferred embodiment polyesters of poly (lactic-co-glycolic) acid (PLGA) are used. These polymers are approved for parenteral administration by the FDA. Because PLGA degrades via non-enzymatic hydrolysis in the initial stages, in vivo degradation rates can be predicted from in vitro data. PLGA degrades to lactic and glycolic acids, substances found naturally in the body.

However, polyesters may be the polymer system of choice for some embodiments. When the polyester material has broken down to molecular weights of about 5000 Daltons, the material may be taken up by cells, including macrophages, so some inflammation may be associated with the breakdown of these polymers.

Copolymers with amino acids may be synthesised, for example glycolic acid and glycine, or lactic acid and lysine (Barrera et al (1993) J Am Chem Soc 115,11010-11011 and Cook et al (1997) J Biomed Mat Res 35, 513-523). These may be useful for immobilising other molecules, for example via the lysyl s-amino moieties. These polymers may be used to attach peptides to surfaces using covalent bonds. For example, peptides may be attached to poly (lactic acid-co-lysine) using 1,1'-carbonyl- diimidazole (CDI, Aldrich) as a linking agent as described in the above references.

By manipulating the molar ratio of lactic and glycolic acid and the molecular weight of the copolymers, different degradation patterns can be obtained. Poly-L-lactide has a degradation time in vitro of months to years. The long degradation time is due to its high crystallinity which protects the polymer from water penetration. Poly-glycolide has a degradation time of one to several months, whereas poly-D, L-lactide is amorphous and has a degradation time of one to a few months. D, L PLGA has a degradation time

in vitro of weeks to months. As the glycolic acid ratio is increased, the rate of degradation increases. Homopolymers of s-caproic acid can remain intact for 2-3 year periods of implantation.

Preferably, at least one of the phases further comprises a plasticiser, examples of which include polyethylene glycol (PEG), polypropylene glycol, polycaprolactone low molecular weight oligomers of those polymers or conventional plasticisers such as those used extensively for commodity plastics materials which include but are not limited to adipates, phosphates, phthalates, sabacates, azelates and citrates. Plasticisers which are the same as the polymers used to form the first or second phases such as poly lactides, lactide-co-glycolide etc may also be used.

The second phase will generally comprise the tissue cells necessary to form the tissue scaffold. The cells may be seeded into a particulate material entrained or carried within the second phase.

It is possible to use any animal cell in the tissue scaffold of the present invention, examples of cells which may be used include but are not limited to bone, cartilage, muscle, liver, kidney, skin, or specialised cells such as placental, amnionic, chorionic or foetal cells, stem cells, chondracytes, or reprogrammed cells from other parts of the body such as adipocytes reprogrammed to become cartilage cells.

Particles which may be used in the second phase to contain or introduce the cells may be of the type described in co-pending patent application PCT/GB02/02813.

Where particulate material is used in the second phase, it is preferred that the particles are porous. Preferably, the porosity of the particle is at least 50%, and more preferably of between 70 and 90%. In any event it is preferred that the pore size of the particle is at least sufficient to receive the cells to be held therein.

Generally, the particles will be micro-particles, although where large cells are to be used the particles may be in the mm range.

Ideally, the pore size is of the order of 50 - 100μm diameter. This means that the particle size is generally of the order of 500μm to 1mm diameter. As

can be seen, the overall particle size will be a function of the pore size. However, the pore size is not always a function of the cell size since large pores may be seeded with tiny cells. Use of such particles provides the advantage of ensuring that the overall matrix retains a level of porosity sufficient for cell growth and hence to accommodate the growing tissue.

Factors useful for the promotion of tissue growth and development may be added to either or both phases or may be used to coat the particles. Additionally, different factors may be added to each of the phases or the or each coating. Factors which may usefully be added include but are not limited to epidermal growth factor, platelet derived growth factor, basic fibroblast growth factor, vascular endothelial growth factor, insulin-like growth factor, nerve growth factor, hepatocyte growth factor, transforming growth factors and bone morphogenic proteins, cytokines including interferons, interleukins, monocyte chemotactic protein-1 (MCP-1), oestrogen, testosterone, kinases, chemokinases, glucose or other sugars, amino acids, calcification factors, dopamine, amine-rich oligopeptides, such as heparin binding domains found in adhesion proteins such as fibronectin and laminin, other amines tamoxifen, cis-platin, peptides and certain toxoids. Additionally, drugs, hormones, enzymes, nutrients or other therapeutic agents or factors or mixtures thereof may be added to one or both of the phases. Again, different drugs, hormones, enzymes, antibiotics, nutrients or other therapeutic agents or factors or mixtures thereof may be added to each of the phases.

The tissue formed according to the method of the present invention may be used *in vivo* as implanted tissue or *in vitro* as tissue cultures. For example, the tissues may be used *in vivo* to replace removed diseased, damaged or non-functioning tissues or *in vitro* as a tissue culture. Advantageously, the present invention allows the production or generation of a 3-dimensional culture tissue which is useful as a research tool such as in the study of drug diffusion or uptake or in the use of secretory cells which often require the cells to be in a 3-dimensional arrangement for secretion to occur.

Where the matrix is to be used in a tissue it is preferably introduced to the tissue prior to solidification.

In a preferred embodiment, where the tissue is to be used *in vivo*, it is preferred that the first phase transforms to a solid or semi-solid state at or close to the body temperature of the animal, or at or close to the pH of the appropriate tissue. Alternatively, setting agents may be used to accelerate solidification. In any event, it is preferred that the conditions needed to cause solidification of the first phase are not detrimental to any cells entrained therein.

The present invention also provides a kit for the formation of a tissue scaffolding matrix as hereindescribed.

In one preferred embodiment of the invention, the first phase comprises a polymer having a low glass transition temperature (Tg) or melting point polymer, for example 45°C, and the second phase comprises a polymer having a higher glass transition temperature or melting point, for example >55°C. The first phase is heated above 45°C in order to render the polymer tacky or fully liquefied, the second phase is introduced to the first phase and mixed. The mixture is allowed to cool. Where cells are to be present in the matrix they may be added to the second phase prior to its introduction to the first phase or simply before solidification of the matrix. Either phase may further comprise growth factors or other pharmacologically active compounds to achieve a controlled release effect in use.

The pore structure is formed by gaps between particles of the second phase or by the incomplete liquefaction of the first phase.

In a second embodiment, the matrix is preferably formed by gelation. In this embodiment, the first phase comprises a material which gels in relation to temperature, e.g. agarose, or pH, e.g. acrylimide. The first phase is brought into the liquid state and is then mixed with a non-gelling, preferably solid, second phase. The mixture is allowed to cool. Cells may be added to the second phase prior to mixing with the first, or after mixing but before full gelling of the gel has occurred.

Embodiments of the invention will now be described with reference to and as illustrated by the following examples which are provided by way of example only.

EXAMPLE 1

Temperature Triggered Solidification.

In this example, the first phase comprises poly (ethylene glycol)/poly (DL-Lactide) blend particles (10 wt% polyethylene glycol) and the second phase comprises porous poly (DL-Lactide) particles manufactured by conventional particulate leaching methods. The two components are mixed together (at a range of ratios between 20:80 and 80:20) and then heated to 60°C to produce a malleable material, which is shaped by the surgeon and applied to the defect site. In this example the first phase does not fully liquefy but becomes a 'tacky' semi-solid at the processing temperature (above the polymers glass transition temperature). In another example, the first phase (of a different polymer blend composition) may be fully liquefied (above the polymers melting transition) at 40-60°C, upon which porous particles of the second phase are mixed together with the still liquid first phase. The material is then shaped and applied to the defect site by the surgeon.

EXAMPLE 2

Solidification by Gelation

In this example the first phase is composed of a solution of Pluronics F127 (20 wt% in buffer or media), which undergoes a liquid to gel transition above 25°C. The second phase comprises porous particles of poly (DL-Lactide) manufactured by conventional particulate leaching methods. The two components are mixed (over a large range of possible ratios, for example 100µls of phase 1 with 100mgs of phase 2) and held as a liquid below room temperature. The components are then delivered via injection to the defect site, where the material gels upon reaching 37°C.

EXAMPLE 3

Solidification by Gelation

In this example the first phase is comprised of a solution of fibrinogen (30mg/ml in buffer or media), which is gelled upon addition of thrombin. The second phase comprises porous particles of poly (DL-Lactide) manufactured

by conventional particulate leaching methods. The two components are mixed (over a large range of possible ratios, for example 100µls of phase 1 with 100mgs of phase 2) and held as a liquid in a syringe ready for injection. Upon injection to the defect site they are mixed (using a dual barrel syringe) with a solution of thrombin (yielding a final concentration of 10 Units/ml), which results in the crosslinking and gelation of the first phase.

EXAMPLE 4

Porous Particles.

In this example large porous particles (≥500µm and up to several mms) are produced by conventional salt leaching techniques. Salt is ground using a pestle and mortar, then sieved with the appropriate size fraction being retained. Ideally the size of the salt particles will be 50-100µm. The salt particles are then mixed with poly (DL-Lactide), in either the melt phase or in an appropriate solvent. The loading of salt will be between 50 and 90wt%. The solid monolith of salt/polymer composite (after cooling or solvent extraction) is then processed into large particles either by grinding or cutting. The salt is then leached from the composite by agitating in water for at least 24 hours.

In a further example the salt/polymer composite may be processed by conventional gas foaming techniques using for example supercritical CO_2.

CLAIMS

1. A process for the production of a porous matrix, the process comprising the steps of:
   - bringing a first, carrier phase into a fluid state,
   - introducing a second, suspended phase into the first phase,
   - mixing the first and second phases to ensure the required distribution of the second phase through the first phase is achieved, and
   - allowing the first phase to solidify, with the second phase entrained therein.

2. A process according to claim 1, in which the first phase is tacky.

3. A process according to claim 1 or claim 2, in which the first phase transforms from a fluid state to a solid or semi-solid state on the change of a single parameter.

4. A process according to claim 3, in which the parameter is temperature, pH, introduction of a setting agent, presence/absence of light, ultra-violet curing infra-red curing, or under anaerobic conditions.

5. A process according to any preceding claim, in which the second phase is a solid phase.

6. A process according to any one of claims 1 to 4, in which the second phase is a liquid phase.

7. A process according to claim 6, in which the liquid phase is an emulsion or suspension of particulate material.

8. A process according to claim 7, in which the particulate material is porous.

9. A process according to claim 7 or claim 8, in which the particulate material is porous and in which the porosity of the particle is of at least 50%.

10. A process according to any preceding claim, in which cells are added to one phase.

11. A process according to claim 10, in which the cells are added to the second phase.

12. A process according to any preceding claim, in which first and second phases are similar materials with different solidifying properties.

13. A process according to any preceding claim in which the phases comprise polymers.

14. A process according to claim 13, in which the polymers are selected from poly-lactide, poly-glycolidé, poly-lactide poly-glycolide copolymers, poly-lactide polyethylene glycol (PEG) copolymers, polyethylene glycol polypropylene block co-polymers, natural or synthetic polymers such as silk, elastin, chitin, chitosan, fibrin, fibrinogen, poly(α-hydroxyacids) especially polylactic or polyglycolic acids, poly (anhydrides) or poly(ortho esters). Polymers of polyhydroxy acids including polyhydroxybutyric acid, lactic, glycolic and s-caproic acid, polyanhydrides, polyorthoesters, polyphosphazenes, polyphosphates, polycaprolactone, copolymers of lactic and glycolic acid with polyethylene glycol), poly (p-dioxanone), polypropylene fumarate, polyol/diketene acetals, addition polymers, poly (sebacic anhydride) (PSA), poly (carboxybiscarboxyphenoxyphenoxy hexane) (PCPP), poly

[bis (p-carboxyphenoxy) methane](PCPM), copolymers of SA, CPP and CPM, poly (amino acids), poly (pseudo amino acids), polyphosphazenes, derivatives of poly [(dichloro) phosphazene], poly [(organo) phosphazenes] polymers, co-polymers prepared from the monomers of these polymers, random blends of these polymers or other mixtures or combinations thereof.

15. A process according to claim 13 or claim 14, in which the polymer is biodegradable.

16. A process according to any one of the preceding claims, in which a plasticizer is added to one or both of the phases.

17. A tissue scaffolding matrix, the matrix comprising a first, carrier phase and a second, suspended phase contained within the first phase, the matrix further comprising cells.

18. A tissue scaffolding matrix prepared according to the process of any preceding claim.

19. A tissue scaffolding matrix according to claim 17 or claim 18, in which the second phase comprises the cells.

20. A tissue scaffolding matrix according to any one of claims 17 to 19, in which the cells are seeded into a particulate material entrained or carried within the second phase.

21. A tissue scaffolding matrix according to any one of claims 17 to 18, in which the cells are animal cells.

22. A tissue scaffolding matrix according to any one of claims 17 to 21, in which the cells are mammalian cells.

23. A tissue scaffolding matrix according to any one of claims 17 to 22 in which the cells are human cells.

24. A tissue scaffolding matrix according to any one of claims 21 to 23, in which the cells are bone, cartilage, muscle, liver, kidney, skin, or specialised cells such as placental, amnionic, chorionic or foetal cells, stem cells, chondracytes, or reprogrammed cells from other parts of the body such as adipocytes reprogrammed to become cartilage cells.

25. A matrix according to any one of claims 17 to 24, in which the matrix further comprises factors useful for the promotion of tissue growth and development.

26. A matrix according to any one of claims 16 to 25, in which the matrix further comprises epidermal growth factor, platelet derived growth factor, basic fibroblast growth factor, vascular endothelial growth factor, insulin-like growth factor, nerve growth factor, hepatocyte growth factor, transforming growth factors and bone morphogenic proteins, cytokines including interferons, interleukins, monocyte chemotactic protein-1 (MCP-1), oestrogen, testosterone, kinases, chemokinases, glucose or other sugars, amino acids, calcification factors, dopamine, amine-rich oligopeptides, such as heparin binding domains found in adhesion proteins such as fibronectin and laminin, other amines tamoxifen, cis-platin, peptides and certain toxoids

27. A matrix according to any one of claims 17 to 26, in which the matrix further comprises drugs, hormones, enzymes, antibiotics, nutrients or other therapeutic agents or factors or mixtures thereof in both phases.

23. A tissue scaffolding matrix according to any one of claims 17 to 22 in which the cells are human cells.

24. A tissue scaffolding matrix according to any one of claims 21 to 23, in which the cells are bone, cartilage, muscle, liver, kidney, skin, or specialised cells such as placental, amnionic, chorionic or foetal cells, stem cells, chondracytes, or reprogrammed cells from other parts of the body such as adipocytes reprogrammed to become cartilage cells.

25. A matrix according to any one of claims 17 to 24, in which the matrix further comprises factors useful for the promotion of tissue growth and development.

26. A matrix according to any one of claims 16 to 25, in which the matrix further comprises epidermal growth factor, platelet derived growth factor, basic fibroblast growth factor, vascular endothelial growth factor, insulin-like growth factor, nerve growth factor, hepatocyte growth factor, transforming growth factors and bone morphogenic proteins, cytokines including interferons, interleukins, monocyte chemotactic protein-1 (MCP-1), oestrogen, testosterone, kinases, chemokinases, glucose or other sugars, amino acids, calcification factors, dopamine, amine-rich oligopeptides, such as heparin binding domains found in adhesion proteins such as fibronectin and laminin, other amines tamoxifen, cis-platin, peptides and certain toxoids

27. A matrix according to any one of claims 17 to 26, in which the matrix further comprises drugs, hormones, enzymes, antibiotics, nutrients or other therapeutic agents or factors or mixtures thereof in both phases.

28. A matrix according to any one of claims 17 to 25, in which each phase of the matrix comprises different drugs, hormones, enzymes, antibiotics, nutrients or other therapeutic agents or factors or mixtures thereof.